REMARKS

Upon entry of the amendment, claims 201, 209, 212-214, 239 and 244 are pending in the application. Claim 238 is cancelled without prejudice. Applicants reserve the right to pursue the subject matter of this claim in one or more continuing applications. Claims 201 is amended and claim 245 is added. Support for the amendments to claim 201 and for new claim 245 is found throughout the instant specification, for example in Example 1. No new matter is added.

Applicants submit herewith a Supplemental Information Disclosure Statement including the references recited in support of the remarks *infra*.

Claim Objections

The Examiner has objected to claim 238 under 37 CFR 1.75(c) for failing to limit the subject matter of independent claim 201. Claim 238 is canceled. Thus, this rejection is moot and should be withdrawn.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 201, 209-214, 238 and 239 under 35 U.S.C. 112, first paragraph as lacking proper enablement in the instant specification. Applicants traverse the rejection with respect to the pending claims as amended herein.

The Examiner has alleged that the neither the art of record nor the instant specification provide any guidance as to how to practice the claimed method in the presence of any copper chelator capable of reducing intracellular available copper concentration to any level in the hematopoietic stem cell. The Examiner, citing Peled et al (Exp. Hematol. 2004;32:547-55), asserts that only TEPA in the presence of the correct combination of cytokines results in effective expansion of hematopoietic stem cells from mononuclear cells. Applicant disagrees.

Contrary to the Examiner's assertions, the instant inventors have repeatedly demonstrated that the inhibition of differentiation of hematopoietic stem and early progenitor cells by copper chelators is the result of reduction of intracellular available copper concentration, and is not restricted to a specific chelator. Moreover, Applicants respectfully submit that the Examiner has misconstrued the teachings of Peled et al., (Exp.

Hematol. 2004;32:547-55). This manuscript clearly teaches that other linear polyamine chelators capable of reducing intracellular copper have the same effect as TEPA on cultured hematopoietic stem cells:

> "The effect of other linear polyamine Cu chelators, such as tetraethylentetramine and pentaethylenhexamine, on the ex vivo expansion of CD34 cells was similar to that of TEPA." *See*, Peled et al. Exp Hematol 2004;32:547-555, page 552

Further, Table 3 of US Patent No. 6,962,698 to Peled et al, issued August 11, 2005 details a number of transition metal chelators, in addition to TEPA, which are capable of reducing intracellular copper, and illustrates the correlation between the reduction in intracellular copper correlates and the inhibition of differentiation of the treated cells.

In an additional post-filing publication, Peled et al. (Exp Hematol. 2005;33:1092-1100) (courtesy copy enclosed) provides further evidence for the role of intracellular available copper concentration in regulating cell differentiation:

> "The results of the present study support the notion that reduction of the chelatable Cu pool rather than a specific TEPA-Cu chelate mediates the mechanism of TEPA's activity on CD34+ cells." *See*, Peled et al, Exp Hematol. 2005;33:1092-1100, page 1099.

Based on the foregoing, Applicants submit that one of ordinary skill in the art would readily recognize that any copper chelator capable of reducing intracellular copper can be used with the present invention and that the skilled artisan would not require undue experimentation to practice the claimed invention

Thus, Applicants submit that the pending claims, as amended herein, are fully enabled by the instant specification such that one of ordinary skill in the art could make and use the invention without undue experimentation. Reconsideration and withdrawal of the present rejection are respectfully requested.

35 U.S.C. § 112, Second Paragraph, Rejections

Claims 201, 209, 212-214, 238 and 239 are rejected under 35 U.S.C. 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the

subject matter which Applicants regard as the invention. Claim 238 is cancelled. Claim 201 (from which the remaining claims subject to the rejection depend) is amended. Applicants traverse the rejection with respect to the pending claims as amended herein.

The Examiner has rejected claim 201 for reciting "and/or" asserting that this term renders the claim unclear. Claim 201 is amended herein to recite "and." Therefore, this rejection is moot and should be withdrawn.

The Examiner has also rejected claim 201 for reciting "said hematopoietic stem cells" asserting that there insufficient antecedent basis for this limitation in claim 201. Applicants disagree.

Claim 201 recites at lines 1-3 "method of expanding an *ex-vivo* population of CD34+, CD34+/CD38- and CD 133+ hematopoietic stem cells, while at the same time, inhibiting differentiation of the hematopoietic stem cells..." (emphasis added). Thus the recitation of "said hematopoietic stem cells" in lines 8 and 11 of claim 201 have proper antecedent basis. Reconsideration and withdrawal are respectfully requested.

35 U.S.C. § 103 Rejection

Claims 201, 209, 212-214 and 238-239 are rejected under 35 U.S.C. 103(a) as being unpatentable over Feitz et al., Bone Marrow Transplant 1999;23:1109-15 ("Feitz") or Wang et al., Sheng Wu Gong Cheng Xue Bao 2002 18:343-7 ("Wang") and WO99/40783 to Peled ("Peled").

The Examiner states that Feitz teaches a method of expanding CD34+ hematopoietic cells from unseparated mononuclear cells, and that there is significant increase of CD34+ cells in the mononuclear cell fraction after one week in culture. The Examiner also states that Wang teaches expanding hematopoietic stem/progenitor cells from mononuclear cells by culturing in a medium containing cytokines, resulting in expansion of the mononuclear cells up to 4 weeks and increasing colony density and proportion of CD34+ cells for 7 days. Finally, the Examiner states that Peled teaches the expansion of hematopoietic stem cells by using a copper chelator, such as TEPA. Thus, the Examiner asserts that it would have been obvious for one of ordinary skill in the art to modify the MNC culturing methods of Fietz or Wang in the presence of a copper chelator as taught by Peled to expand CD34+ cells from unselected mononuclear cells with a reasonable expectation of success. *See*, Office Action

at pages 10-12. Applicants traverse the rejection with respect to the pending claims as amended herein.

Claim 201 (from which the remaining claims subject to the rejection depend) is amended to recite "...culturing said mononuclear cells *ex-vivo* for a period greater than 7 days under conditions allowing for cell proliferation... thereby expanding a population of said hematopoietic stem cells while at the same time inhibiting differentiation of said hematopoietic stem cells *ex-vivo* for a period greater than 7 days."

These features are not taught or suggested by the combination of Feitz or Wang and Peled.

Moreover, Applicants submit that the skilled artisan would not combine the teachings of Feitz or Wang and Peled to reach the present invention with a reasonable expectation of success.

Specifically, Applicants assert that the state of the art at the time of filing of the instant application clearly indicated the difficulty in expanding hematopoietic stem and/or early progenitor cells from unselected mononuclear cell fractions. For example, McNeice et al., Cytotherapy 2004;6:311-17 (courtesy copy enclosed) describes the failure of expansion of hematopoietic stem cells from unselected cord blood mononuclear cells:

> "CB MNC products were placed into ex vivo expansion culture without CD34 selection and, as demonstrated in Figure 1, failed to increase cell numbers. In fact, fewer total nucleated cells (TNC) were recovered after 14 days of culture than seeded on day 0. This result is consistent with previous studies that reported that unselected CB cells failed to expand in growth factor-stimulated cultures [4]. In contrast, CB MNC seeded onto preformed layers of MSC resulted in significant expansion of TNC (Figure 1). *See*, McNeice et al, at page 313-314.

Further, McNeice et al. references Briddell et al, J Hematother 1997;6:145-50 (courtesy copy of the Abstract enclosed) at reference [4] and Briddell et al emphatically states that selection of CD34+ cells is necessary prior to expansion of CFU cells from unselected mononuclear cells:

> "...we evaluated the potential of UCB cells for their ability to expand granulocyte-macrophage colony-forming cells (GM-CFC)

> and burst-forming unit-erythroid (BFU-E) cells over 10 days...Either unselected UCB cells or CD34+ UCB cells, selected with Magnetic Activation Cell Sorting technology (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany), were incubated for 10 days at 37 degrees C without refeeding. Unselected UCB cells seeded at 1 X 10(6)/ml produced an average expansion of 1.4-fold in total cells, 0.8-fold in GM-CFC, and 0.3-fold in BFU-E cells. By contrast, CD34+ selected UCB cells seeded at 1.0 X 10(4)/ml produced an average expansion of 113-fold in total cells, 72.6-fold in GM-CFC, and 49-fold in BFU-E cells. These data demonstrate that CD34+ cell selection is necessary for optimal expansion of both GM-CFC and BFU-E cells." *See*, Briddell et al., at the Abstract.

Feitz does not provide any solution to the challenge of expansion of hematopoietic stem/progenitor cells from unselected mononuclear cells. As noted by the Examiner, Feitz failed to observe any significant increase in total or CD34+ cells with greater than 7 days culture of the unselected mononuclear cells, as requied by the instant claims. Yet further, Feitz teaches that MNC fraction did not profit from refeeding and reseeding, and only proliferated in static culture conditions. *See*, Feitz at page 111. Significantly, Feitz teaches away from the present invention in describing the expansion of predominantly committed cells from culture:

> "This indicates that the increase in CD34+ cells is not so much because of expansion of the early but rather late progenitor pool" *See*, Feitz at page 1114/

As stated by the Examiner, Wang only teaches an increase in colony density and proportion of the stem and/or progenitor fraction for only the first 7 days of culture. Long term culture potential, defined as the ability of cells to generate myeloid clonogenic progeny in long term cultures for a minimum of 5 weeks, has long been recognized in the art as a significant indicator of stem and/early progenitor cell content of a cultured cells, critical to engraftment and self-renewal in a transplanted cell population. *See*, for example, Petzer et al, PNAS 1996;93:1470-74 and Reya, T., Rec Prog Horm Res 2003;58:283 (courtesy copies enclosed).

Thus, the failure of the mononuclear cell cultures of Feitz and Wang to produce and increase the numbers of clonogenic cells for a period of greater than 7 days indicates that there is there is no true expansion of hematopoietic stem and/or early progenitor cells, as required by the instant claims, but, as concluded by Feitz, most likely the gradual maturation and differentiation of existing, committed late progenitors.

In stark contrast to the cultures of Feitz and Wang, the claimed methods have been shown effective in providing expansion of hematopoietic stem and early progenitor cells (CD34+ and CD34+/38-) from unselected mononuclear cultures, while simultaneously inhibiting differentiation thereof, for periods of greater than 7 days, two weeks, 10 weeks and up to 12 weeks. *See*, for example, Example 1, Figs. 1a-c, 2, and 3 of the instant specification. Unselected mononuclear cell cultures supplemented with a copper chelator showed highly significant long term expansion of CD34+ cells and the rare stem/early progenitor cells (CD34+38-) as compared with minimal expansion of these cells obtained in cultures treated without a copper chelator:

> "The results, illustrated in Figures 1a-b, 2 and 3, show that addition of TEPA chelator to non-purified MNC cultures, substantially and progressively increased the number of $CD34^+$ cells, $CD34^+$ colony-forming cells and $CD34^+CD38^-$ cells, over a 12-week period. Thus, in MNC cultures treated with TEPA, the cumulative number of $CD34^+$ cells increased from a non-detectable level to over 8×10^7 cells/ml, after 2 and 12 weeks, respectively (Figures 1a-b); the cumulative number of $CD_{34}{}^+CD_{38}{}^-$ cells increased from a non-detectable level to 2.5×10^7 cells/ml, after 2 and 12 weeks, respectively (Figure 2); and the number of $CD34^+$ CFUs increased from a non-detectable level to 3.2×10^7 cells/ml after 2 and 10 weeks, respectively (Figure 3). On the other hand, when TEPA was not added to MNC cultures (untreated controls), no significant expansion of stem or progenitor cells was measured throughout the 12-week period. Furthermore, the stem and progenitor cells densities in the TEPA-treated MNC cultures, either equalized or surpassed the densities of stem and progenitor cells in pre-purified $CD34^+$ cell cultures (not treated with TEPA, positive controls). Morphological analysis of cells derived from long-term and TEPA-treated MNC cultures, revealed a high proportion of non-differentiated cells, while most of the cells derived from long-term and MNC cultures not treated with TEPA, where fully differentiated. *See*, page 91-92 of the instant specification.

Thus, Feitz and Wang fail to teach the *ex-vivo* expansion of hematopoietic stem and/or early progenitor cells from unselected mononuclear cells in the presence of early and late acting cytokines for greater than one week, as required by the instant invention. Peled does not cure these deficiencies of Feitz and Wang. Peled does not teach or suggested unselected mononuclear cells and does not teach or suggest the expansion of CD34+ cells from an unselected MNC population.

As such, Applicants submit that the combination of Feitz or Wang and Peled fail to teach or suggest all the limitations of the claimed invention, and as such, do not constitute prima facie evidence for obviousness. Further, Applicants submit that one of ordinary skill in the art reading the combination of Feitz or Wang and Peled would have no reasonable expectation of success in reaching the claimed invention. Reconsideration and withdrawal are respectfully requested.

Double Patenting Rejections

The Examiner has rejected claims 201, 209, 212-214, 238-239 and 244 as not being patently distinct over claims 1-11 of U.S. Patent No. 7,169,605. Claims 201, 209, 212-214, 238-239 and 244 have been provisionally rejected as not being patently distinct over claims 1-6, 8-17, 19-22, 123-131 of copending U.S. Patent Application No: 10/418,639 and claims 1, 2-11 and 23 of copending U.S. Patent Application No: 10/564,777. Applicants will consider filing a terminal disclaimer upon notice of allowable subject matter in this application.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance. Should any questions or issues arise concerning this application, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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